AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph on page 14, line 21 to page 15, line 4, under the section heading "**Brief Description of the Drawings**," with the following paragraph:

--FIG. 1. Depiction of a single crossover recombination event resulting in integration of a plasmid into the bacterial chromosome. Isolation of such recombinants indicates that the targeted gene is not essential.

FIG. 2. Single crossover and integration of a plasmid resulting in the replacement of a wild type promoter with a regulatable promoter ("promoter swap" strategy).

FIGS. 3-5 respectively contain schematics of plasmids pMOD, pMOD (Erm-1) and pMOD (Cm).

~~FIGS. 6-8 respectively contain the sequences for pMOD, pMOD (Erm-1) and pMOD (Cm).~~

FIG. 6 contains the sequence for pMOD (SEQ ID NO: 11).

FIG. 7 contains the sequence for pMOD (Erm-1) (SEQ ID NO: 12).

FIG. 8 contains the sequence for pMOD (Cm) (SEQ ID NO: 13).--

Please replace the paragraph on page 15, lines 6-18, with the following paragraph:

The essential open reading frames identified in the present invention are set forth in Table 1. These open reading frames were originally part of a library of putative nucleic acid sequences generated from *S. aureus* strain. The sequence of staph col, a staph aureus strain similar to RN4220, is available at ~~http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gsa~~ the web address having the domain name tigr.org and pathname tigr-scripts/CMR2/GenomePage3.spl?database=gsa, which sequence is incorporated herein by reference. The SA Numbers in Table 1 correspond to the Tigr number system. RN4220. Nevertheless, it is expected that the genes identified will be also be essential or important in related *S. aureus* strains as well as other Staphylococcus species, given the low sequence diversity that exists between *S. aureus* strains of widely diverse environments and the pronounced structural and functional homology of gene products. Thus, it is expected that

agents identified as antibacterial based on their interaction with genes or gene products *S. aureus* will be broadly applicable as antibacterial agents against a variety of Staphylococcus species as well as other bacteria including but not limited to *Escherichia, Hemophilus, Vibrio, Borrelia, Enterococcus, Heliobacter, Legionella, Mycobacterium, Mycoplasma, Neisseria, Pseudomonas, Streptococcus,* etc.

Please replace the paragraph on page 23, lines 15 to page 25, line 5, with the following paragraph:

--The immunogenic composition may further comprise a parenteral adjuvant. Parenteral adjuvants suitable for use in the invention include: (A) aluminum compounds (*e.g.* aluminum hydroxide, aluminum phosphate, aluminum hydroxyphosphate, oxyhydroxide, orthophosphate, sulfate *etc.* (*e.g.* see chapters 8 & 9 of *Vaccine design: the subunit and adjuvant aproach*, eds. Powell & Newman, Plenum Press 1995 (ISBN 0-306-44867-X) (hereinafter "*Vaccine design*"), or mixtures of different aluminum compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous *etc.*), and with adsorption being preferred; (B) ~~MF59~~ MF59™ (5% Squalene, 0.5% ~~Tween 80~~ TWEEN®-80 (polyoxyethylenesorbitan, monooleate), and 0.5% ~~Span 85~~ SPAN®85 (sorbitan trioleate), formulated into submicron particles using a microfluidizer) (*see* Chapter 10 of *Vaccine design; see also* International patent application WO 90/14837); (C) liposomes (see Chapters 13 and 14 of *Vaccine design*); (D) ISCOMs (*see* Chapter 23 of *Vaccine design*); (E) SAF, containing 10% Squalane, 0.4% ~~Tween 80~~ TWEEN®-80 (polyoxyethylenesorbitan, monooleate), 5% ~~pluronic block polymer L121~~ PLURONIC™L121 (block copolymer of propylene oxide and ethylene oxide), and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion (*see* Chapter 12 of *Vaccine design*); (F) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% ~~Tween 80~~ TWEEN ®-80 (pol yoxyethylenesorbitan, monooleate), and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+CWS (Detox™); (G) saponin adjuvants, such as QuilA or QS21 (*see* Chapter 22 of *Vaccine design*), also known as Stimulon™; (H) ISCOMs, which may be devoid of additional detergent (International patent

application WO 00/07621); (I) complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA); (J) cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, tumor necrosis factor, *etc.* (*see* Chapters 27 & 28 of *Vaccine design*); (K) microparticles (*see* above); (L) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) (*e.g.* chapter 21 of *Vaccine design*); (M) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (European patent applications 0835318, 0735898 and 0761231); (N) oligonucleotides comprising CpG motifs (*see* Krieg (2000) *Vaccine*, 19:618-622; Krieg (2001) *Curr. Opin. Mol. Ther.*, 2001, 3:35-24; WO 96/02555, WO 98/16247, WO 98/18810, WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581, etc.) *i.e.* containing at least one CG dinucleotide, with 5-methylcytosine optionally being used in place of cytosine; (O) a polyoxyethylene ether or a polyoxyethylene ester (International patent application WO 99/52549); (P) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (International patent application WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (International patent application WO 01/21152); (Q) an immunostimulatory oligonucleotide (*e.g.* a CpG oligonucleotide) and a saponin (International patent application WO 00/62800); (R) an immunostimulant and a particle of metal salt (International patent application WO 00/23105); (S) a saponin and an oil-in-water emulsion (International patent application WO 99/11241); (T) a saponin (*e.g.* QS21)+3dMPL+IL-12 (optionally+a sterol) (International patent application WO 98/57659); and (U) other substances that act as immunostimulating agents to enhance the effectiveness of the composition (*e.g.* see Chapter 7 of *Vaccine design*).--

Please replace the paragraph on page 23, line 6, with the following paragraph:

--Aluminium compounds and ~~MF59~~ MF59™ are preferred adjuvants for parenteral use.--

Please replace the paragraph on page 42, lines 17 to page 43, line 14, with the following paragraph:

--TN5 transposons are prepared using EZ::TN™pMOD<MCS> Transposon Construction Vector and EZ::TN™ Transposase (Epicentre Technologies, Madison, Wis.). Initially two separate transposomes are designed using either chloramphenicol or erythromycin markers. Although both are successful in producing transposon mutants, the majority of the library is the result of the erythormycin transposon as it produces more mutants per electroporation. The choloramphenicol marker is amplified from plasmid pC194 and cloned into the pMOD™<MCS>. Amplifications from pC194 are performed using the primers Cm194-HindF (5'-TATATaagcttGTTACAGTAATATTGACTTT-3') (SEQ ID NO: 1) and Cm194-KpnR (5'-TAACGggtaccGTrAGTGACATTAGAAAACC-3') (SEQ ID NO: 2). The erythromycin marker is amplified from plasmid pTLV-1 using the primers Erm917-HindF (5'-AAATaagcttTAGAAGCAAACTTAAGAGTG-3') (SEQ ID NO: 3) and Erm9117-KpnR (5'CGGTCGTTATggtaccATTCAAATTTATCC-3') (SEQ ID NO: 4). Each primer contains a restriction enzyme site, designated in lower case above, for cloning. The antibiotic markers are amplified from their respective plasmids under the following conditions: 94°C for 1 minute followed by 30 cycles of 94°C for 1 min 30 sec, 60°C for 45 sec and 72°C for 1 min with a final extension time of 5 min. The markers are then cloned into the MCS of plasmid pMOD™<MCS> Transposon Construction Vector. The transposon is then removed from the pMOD backbone by digestion with PvuII and run on an agarose gel. The DNA is purified from the agarose using QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, Calif.). 100 ng per microliter is generally obtained. Transposomes are made by mixing 500ng of the purified transposon DNA with 5 ul of sterile water or (10 mm TRIS, pH8), 5 Units of EZ::TN™ Transposase (Eppicentre Technologies, Madison, Wis.) and 5 ul of 100% glycerol. The transposome reaction is mixed and incubated at room temperature for 30 minutes. 2 microliters of the transposome mixture is electroporated per aliquot of electrocompetent cells.--

Please replace the paragraph on page 44, line 21 to page 45, line 17, with the following paragraph:

--The techniques used to characterize the DNA sequence of the transposon mutants consists of two PCR reactions were previously described by Kolter et al. [3]. For the first round

of amplification, 5 ul of the InstaGene Lysis suspernatant is used as the template. In the first round of amplification, the primer unique to the transposon TNErm-1R (5'CTGTTTCAAAACAGTAGATG-3') (SEQ ID NO: 5) is used for the Erythromycin transposon and TNCm-1R2 (5'GATAGGCCTAATGACTGGC-3') (SEQ ID NO: 6) is used for the Chloramphenicol transposon with arbitrary primer arb-8 (5'-GGCCACGCGTCGACTAGTACNNNNGATAT-3') (SEQ ID NO: 7). This first amplification conditions are 1 minute at 94°C, followed by 6 cycles (30 seconds at 94°C, 30 seconds at 30°C, 2 minutes at 72°C) and 30 cycles (30 seconds at 94°C, 45 seconds at 45°C, 2 minute at 72°C). The first PCR products are used for the second amplification. The primers used in the second are TNErm-2R (5'CAACATGACGAATCCCTCCTTC-3') (SEQ ID NO: 8) or TNCm-2R2 (5'-GTCGGTTTTCTAATGTCACTAACG-3') (SEQ ID NO: 9) for the erythromycin or chloramphenicol transposons respectively, plus an arbitrary primer arb-tail (5'-GGCCACGCGTCGACTAGTAC-3') (SEQ ID NO: 10). For the second, PCR, 5 ul from the first amplification round are used for template. The amplification conditions for the second PCR were 1 minute at 94°C followed by 30 cycles (30 seconds of 94°C, 45 seconds at 50°C and 1 minutes at 72°C). The PCR product from the second amplification was purified prior to sequencing by treatment with S1 nuclease and Shrimp Alkaline Phosphatase SAP (Roche). For this, 100 ul sinculease/SAP was added to 10 ul PCR product. The S1/SAP mixture was incubated at 37°C for 20 minutes followed by a 15 minute incubation at 80°C. 7 ul of the S1/SAP products were sequenced on an ABI 377 using the primer from the secondary PCR, TNErm-2R or TNCm-2R2.—

Please replace the paragraph on page 46, line 14 to page 47, line 9, with the following paragraph:

--Transposon insertions are generated using the above-described methods in S. aureus. The pMOD, pMOD (Erm-1) and pMOD (Can) plasmids referred to in the described methods are contained in FIGS. 3, 4 and 5 respectively. The sequences for these plasmids are contained in FIG. 6 ~~(SEQ ID NO: 1)~~ (SEQ ID NO: 11), FIG. 7 ~~(SEQ ID NO: 2)~~ (SEQ ID NO: 12) and FIG. 8 ~~(SEQ ID NO: 3)~~ (SEQ ID NO: 13) respectively also available at

~~www.epicentre.com/sequences.asp Epicentre DNA sequences~~ the web address having the domain name epicentre.com and pathname seq uences.asp Epicentre DNA sequences. Using these methods >7400 transposon mutants are generated.

Please replace the paragraph on page 47, line 19 to page 48, line 4, with the following paragraph:

--With every insertion added to the map, the regions of the genome containing essential genes, and particularly those containing operons containing essential genes (because of potential polar effects of insertions in upstream genes), begin to become apparent because these regions will not be able to accommodate transposon insertions. Table 1 shows a listing of the open reading frames identified as existing between transposon insertions, with an assigned probability of essentiality according to the length of the putative open reading frames. These open reading frames cane be subjected to further analysis. For instance, the predicted ORFs can be examined individually for (1) identity with known genes of S. aureus with sequences deposited in ~~GenBank~~ GENBANK® (genetic sequence database), (2) similarity with well-characterized genes from other bacteria, or (3) presence of known functional motifs.--